Biosciences, Biotechnology Research Asia Vol. **5**(2), 545-550 (2008)

Antimalarial activity of *Andrographis paniculata* in mice

B.A.H ZAINAL-ABIDIN and L. JANG SING

School of Bioscience and Biotechnology, Faculty of Science and Technology
Universiti Kebangsaan Malaysia, UKM – 43600 Bangi, Selangor Malaysia

(Received: July 20, 2008; Accepted: October 21, 2008)

ABSTRACT

The antimalarial activity of the capsule of dried whole plant parts of herb *Andrographis paniculata* against *Plasmodium berghei* in male ICR mice was examined. In this study four concentrations of aqueous extract (AE) of the herb i.e 50, 100, 200 and 400 µL/kg body weight (BW) were given to mice either orally or intraperitoneally (i.p.). Infected mice without treatment and infected mice treated with anti-malarial drug chloroquine diphosphate (10mg/kg BW) were used as negative and positive controls respectively. A dose of 1.0×10^6 *Plasmodium berghei* - infected red blood cells injected i.p.ly was used to initiate infection in mice. Thin - and Giemsa's stained – blood smears were prepared to determine parasitaemias. Results from the four-day suppressive antimalarial tests showed that all four concentrations of AE given orally had varying degrees of antimalarial activities against the parasites. The 200 µL/kg BW dose caused the highest suppressive activitiy i.e. 77.76% as compared to 68.9%, 58.86% and 43.81% caused by the 100, 400 and 50µL/kg BW doses respectively. Results in the i.p treatments of AE also showed almost similar pattern of infections but with inferior implications and that the 200 µL/kg BW dose still caused the highest degree of suppression (68.74%). This dose caused the lowest peak of parasitaemia on D+4 i.e. 1.2 ± 1.650 % as compared to 3.85 ± 0.240 % in the negative control group and more than 2.0 % in the rest of the treated groups. Results from the prophylactic antimalarial activity tests evidently proved that the 200 µL/kg BW dose caused about 1.7 folds higher degree of suppression to malarial parasitaemias (82.46%) as compared to the 100 µL/kg BW dose (53.07%) and closer to the value shown by the positive control group (94.08%). All these results strongly show that the AE extracts of *A. paniculata* have antimalarial activity against *P. berghei* in mice and that the capsule used in this still retains this activity.

Key words: suppressive antimalarial activity, *Andrographis paniculata*, aqueous extract (AE), rodent - malaria, *Plasmodium berghei*

INTRODUCTION

The importance of traditional or herbal medicine for treating various diseases including malaria was recognized by WHO since late 1970's. Many communities living in tropical regions used local flora as a means of preventing and treating malaria. There are more than 1000 plants species[1] used to treat malaria throughout the world and up to 80% of patients choose to use traditional medicines to treat malaria largely due to their affordable price and readily available[2].

Andrographis paniculata (king of bitter) is one of the most known herbal plants which has long been used in traditional Chinese, Indian and Malay herbal medicine[3-5]. Its extracts or dried forms in capsule were used to treat digestive problems, snakebite and infections ranging from dysentery to malaria[3-5]. The major constituents of this plant is diterpene lactones or andrographolides with immune-stimulating properties[6]. This compound was also able to decrease viral load and increase CD4 lymphocytes in patients with HIV infection[7] and helping to reduce symptom severity in people with

common colds[8]. In Malaysia *A. paniculata* is one of the herbal medicinal plants which are also widely used by the traditional herbal practitioners or healers for treating high blood pressure and diabetes in particular and that the locals are encouraged to plant this herb at small or even commercial scales[5] for local needs or for exports. In light of its broad acceptance by many societies, a study was conducted to examine the antimalarial activity of capsule of this plant's extracts produced by a local firm against a rodent-malaria model in mice.

EXPERIMENTAL

Experimental animals

Eight-week-old male ICR strain white mice (25-30g body weight (BW) were obtained form the Animal House, Universiti Kebangsaan Malaysia. They were kept in standard propylene cages and acclimatized for two weeks before the experiment. They were fed with standard commercial mouse-pellets and given drinking water *ad libitum*. The handling for experimental usage of this animal was in accordance with the Universiti Kebangsaan Malaysia Animal Ethical Committee (UKMAEC) Guidelines[9].

Inoculation of malaria parasite

Rodent-malaria parasite *Plasmodium berghei* (pzz1/00) obtained from the Parasitology Lab, School of Bioscience and Biotechnology, Universiti Kebangsaan Malaysia was maintained in ICR mice by intraperitoneal (i.p) injection of infected blood into clean mice weekly. Parasite inoculum was prepared from the blood of infected donor mouse having about 20% parasitaemia diluted serially in Alsever's solution. An inoculum of 1.0×10^6 *Plasmodium berghei* - infected red blood cells in 0.ml suspension was used as standard dose and injected i.p.ly into each mouse to initiate infection.

Administration of extracts

Dried herb (from whole *A. paniculata* plant parts) in capsules prepared by IMED LAB Sdn Bhd Penang Malaysia was used in this study. Ten (10) grams of the dried herb were used and added to 100ml distilled water, mixed up and left at room temperature for one hour. Clear yellow solution was obtained by filtering out the suspension with filter paper. This aqueous solution was taken as stock solution and kept in tight-bottle until used. The stock solution was diluted in buffered mammalian saline to give four different dosages viz. 50, 100, 200 and 400 ìL/kg body weight (BW) as described by Abdulelah and Zainal-Abidin[10]. These aqueous dosages were administered orally or i.p.ly into mice as a means of treatment.

Evaluation of antimalarial activity

Antimalarial activity of the aqueous extract was assessed by employing techniques described by Peters[11] and Misra et al.[12]. In the four-day suppressive test, mice were divided into groups of six animals and treatments with the extract were given for four consecutive days from the day of the infection of the parasite i.e. from DO to D+3. Parasitaemia (percent of parasitized red blood cells, rbc) was determined on D+4 from the tail blood and continued alternate days until the death of the animal. Mice received infection without treatment were used as negative control whereas mice received infection and treated with antimalarial drug chloroquine diphosphate (10mg/kg BW) served as positive control.

Mode of treatment and the dose which give the highest degree of suppression of parasitaemia in the four-day suppressive test were used in the prophylactic activity test in which mice were given preinfection treatment for four days (D-4 to D-1) and injected with the parasite on D0. Parasitaemia was determined on D+3 and continued alternate days until the death of the animal. Negative and positive control groups were also employed in this test.

Experiments were performed both for i.p. and oral treatments of the aqueous extract.

Analysis of results

The percentage of parasitaemia was estimated from thin blood smears prepared from tail blood and stained with the Giemsa stain. Using microscope at x100 magnification, the number of parasitized red blood cells out of 5000 cells in random fileds.were counted. The parasitaemia was estimated as follows:

(Number of infected red blood cells ÷ Total number of red blood cells observed) x 100.

The mean of percent of inhibition or suppression[17] = 100 - [(Pt x 100) ÷ Pc]

Pt = parasitaemia in treated mice
Pc = parasitaemia in control mice.

Statistical analysis

The results were expressed as mean ± SD. Data were analyzed using Student t-test and one-way ANOVA where appropriate. Values of $p< 0.05$ was taken as significant.

RESULTS

Four-day suppressive antimalarial activity of *A. paniculata*

Results of the present study indicated that all four different doses of the aqueous extracts (AE) of *A. paniculata* given orally showed varying degrees of suppressive antimalarial activities against *P. berghei* in mice (Table 1). The 200 µL/kg BW dose caused the highest suppressive activity (77.76%) and followed by the 100 (68.90%), 400 (58.86%) and 50 µL/kg BW (43.81%) doses respectively. The degree of the suppression can be related to the mean parasitaemia achieved in the infected mice on D+4 post-infection. It seems that a four- day treatmens were capable of suppressing the infection to a level less than 2.0% in all 3 higher doses (100, 200 and 400 µL /kg BW respectively) groups whereas the lowest dose (50 µL/mgBW) did not show this capacity and by D+4, parasitaemia was high (more than 2%) paralleled to that of the negative control group (4%) and thus caused the lowest degree of suppression. In comparison positive control group (treated with chloroquine)

Table 1: Mean parasitaemia and suppression of parasitaemia following oral treatments of different dosages of aqueous extracts (AE) of *A. paniculata* in *P. berghei*- infected mice in the four-day suppressive antimalarial activity test

Treatments (oral)	Dose (µL/kg BW)	Mean Parasitaemia ± S.D (%)	Suppression (%)
Aqueous extract AE	50	2.36 ± 1.347	43.81
AE	100	1.306 ± 1.557*	68.90
AE	200	0.934 ± 0.928*	77.76
AE	400	1.728 ± 0.989	58.86
Positive control	10 mg	0.00 ± 0.00	100.00
Negative control	0.1 ml	4.20 ± 1.093	00.00

* significant difference ($P < 0.05$) as compared to the negative control

Table 2: Mean parasitaemia and suppression of parasitaemia following i.p treatments of different dosages of aqueous extracts (AE) of *A. paniculata* in *P. berghei*- infected mice in the the four-day suppressive antimalarial activity test

Treatments (oral)	Dose (µL/kg BW)	Mean Parasitaemia ± S.D (%)	Suppression (%)
AE	50	2.45 ± 2.251	36.4
AE	100	2.08 ± 1.887	45.95
AE	200	1.20 ± 1.650*	68.74
AE	400	2.54 ± 0.885	34.01
Positive control	10 mg	0.00 ± 0.00	100.00
Negative control	0.1ml	3.85 ± 0.240	00.00

* significant difference ($P < 0.05$) as compared to the negative control

caused 100% suppression at 10mg/kg BW. The degree of the suppression was also evident from the fact that all treated groups had delayed pre-patent periods of between 3-4 days and that negative control group had pre-patent period lasted for 2 days only. On the other hand the positive control group had pre-patent period lasted for 8 days. Comparison in survival periods between all groups showed that although the AE treatments did not guarantee total protection from the infection, but most of the infected mice survived much longer than the negative control group (for example the 100 and 200µL/kg BW groups had survival times of 9.20 ±0.837 and 9.40 ± 0.548 days respectively as compared to 7.60 ± 0.548 days in the negative control group, $p<0.05$). Twenty percent of the positive control group died within 15.40 ± 1.14 days post-infection but the rest of the group survived until the end of the experiment.

In the i.p. treatments of the AE, almost similar pattern of infections were observed but with less superior implications (Table 2). The only significant ($p<0.05$) mean parasitaemia on D+4 was shown by the 200 µL/kg BW group (1.20 + 1.650 % as compared with 3.85 + 0.240 % in the negative control) which resulted in the highest suppression rate (68.74%). The 50, 100 and 400 µL/kg BW groups had suppression less than 50% (i.e between 36 t0 46% only). The 200 ìL/kg BW group also had longer and significant survival time (9.20 + 1.342 days, $p<0.05$) as compared to the negative control group.

All these results seem to indicate that oral treatments of the AE were more effective than i.p. treatments in reducing parasitaemia and prolonged the survival time of the *P. berghei* - infected and treated mice.

Prophylactic antimalarial activity of *A. paniculata*

Since the 100 and 200 ìL/kg BW doses given orally produced sinificant degree of suppression against malaria infection in the four-day suppressive test above, they were taken for further tests for their prophylactical activities.

The results (Table 3) showed that AE at 200 µL/kg BW produced 82.46% suppression which was significant ($p<0.05$) when compared with that of the negative control group whereas the 100 µL/kg BW gave only 53.07% suppression. The positive control group had the highest suppression rate of 94.08%. It was evident here that the degree of suppression of parasitaemia correlated with the mean parasitaemia noted on D+3 post-infection of the parasite. The lower the mean parataemia in the infected mice, the higher the degree of suppression that could be expected and *vice versa.*

In conclusion, the AE obtained from capsules of dried whole plant of *A. paniculata* shows antimalarial activity agains the rodent - malaria *P. berghei* parasites in mice.

Table 3: Mean parasitaemia and suppression of parasitaemia following oral treatments of 100 and 200 µL/kg BW respectively of aqueous extracts (AE) of *A. paniculata* in *P. berghei*- infected mice in the prophylactic antimalarial activity test

Treatments (oral)	Dose (µL/kg BW)	Mean Parasitaemia ± S.D (%)	Suppression (%)
AE	100	0.76 ± 0.428	53.07
AE	200	0.16 ± 0.219*	82.46*
Positive control	10 mg	0.05 ± 0.096*	94.08*
Negative control	0.1 ml	0.91 ± 0.823	00.00

* significant difference ($P < 0.05$) as compared to the negative control

DISCUSSION

The burden of malaria on world population remain enormous although greater commitments and access to malaria control interventions had increased sharply between 2004 and 2006 and that many malaria-laden countries in Africa had 50% or more, less cases and deaths due to malaria in the same period[11]. Despite these successes however, many poor countries especially in tropical Africa still require or dependent on alternative medicine especially herbal traditional medicines to alleviate and reduce their sufferings. In this context, greater efforts to explore and identify more potential herbal medicines and research into their antimalarial activities are very much encouraged and continued for many years to come. Our present study on *A. paniculata* is one of such effort since this plant is easily propagated in many local conditions of the world especially in tropical climate.

Both of the four-day suppressive and prophylactic tests provided the evidence that AE of *A. paniculata* at 200µL/kg BW produced the most promising results in that it reduced or suppressed malaria infections between 69 to 83% comparable to that of the positive control. It seems that this antimalrial activity is not dose-dependent in nature. Lower doses of AE i.e. 50 and 100 µL/kg BW many not have enough concentrations of bioactive compounds to actively suppress the development of parasites in the blood. Likewise the highest dose i.e. 400 µL/kg BW which also have similar effects, may have other compounds which are not antimalarial in nature but might suppress mechanism(s) which directly affect the host (mice) cells and indirectly the parasites. In a situation whereby the concentration of the non-suppressive compounds are in excess i.e. as in case of the 400 µL/kg BW used in this study, they might act on the host's cells which might then agrravate or promote the infection of the parasites in the blood. This view is in support for the fact that either the methanol- or chloroform extracts (supposedly to contain the active compounds) of *A. paniculata* were found to be non-toxic even at a LD_{50} exceeding 5000mg/kg BW[12] .

The route of treatment of AE into mice may play important role. This study has shown that the oral route was more effective in suppressing malaria infection comparaed to i.p. route. This may be due its readiness to be absorbed in the oral route since it is soluble in water and that it quickly reaches the blood sytem[13]. The effectiveness of AE is attributed to its andrographolide and/or other related compounds in particular neoandrographolide, but the mechanism(s) of action is largely unknown although suggestion have been made that these compounds are immunostimulants to host cells[6,14] . Andrographolide given at 25mg/kg BW suppressed parasitaemia at 85.7% on D+4 post-infection[15] and that neoandrographolide at 2.5mg/kg BW given for 25days pre-infection reduced parasitaemia for 25 days post-infection[16]. It is not known whether the concentrations used in these two studies were equivalent to 200 µL/kg BW as used in the present study, but the fact remains to be seen that either andrographolide alone or in concert with neographolide or *vice versa* which produced the suppressive effect against the parasite.

The results of the present study are strongly in agreement with the results of other studies carried out elsewhere[13,14,15,16] that *A. paniculata* and/or its extracts have antimalarial activities agains rodent-malaria *P. berghei.* It is interesting to note that even crude extract of the whole plant as used in this study still retains its activity. More research must be carried out as to understand the actual mode of action of such extracts and that other research methodologies must be employed to confirm the actual active compounds which have the activity. The other usages of *A. paniculata* or its extracts or products must also be explored for other diseases such as diabetis or high blood pressure. The results of the present study may also indirecly highlight this plant as a very potential herbal species which has wider applications in life.

ACKNOWLEDGEMENTS

The authors wish to record their appreciation for the help and facilities provided by the School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

REFERENCES

1. Willcox, M.L., Cosentino, M.J., Pink, R., Bodeker, G. and Wayling S. "Natural products for the treatment of tropical diseases." *Trends in Parasitology*, **17(2**): 58-60 (2001).
2. Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D and Guo, Z. "Medicinal Plants in therapy." *Bull. WHO,* **63**: 965 (1985).
3. Nadkarni, A.K. and Nadkarni**,** A.M**.** *Indian Materia Medica***.** Popular Prakashan, Bomby **1**: 101-103 (1976).
4. Bensky, D., Gamble, A. and Kaptchuck, T. *Chinese Herbal Medicine.* Eastland Press Seattle, Revised Edition, 95 (1993).
5. Anon. *Penanaman Tumbuhan Ubatan & Beraroma (Planting of Medicinal & Aromatic Plants).* Malaysian Agriculture Research and Development Institute (MARDI), Serdang Malaysia, **7**: 36-47 (2006).
6. Bone, K.*The Story of Andrographis paniculata, a new 'immune system' herb.* Nutrition & Healing (1998).
7. Calabrese, C., Berman, S.H and Babish, JG. "A phase I trial of andrographolide in HIV positive patients and normal volunteers". *Phytotherapy Research*, **14***:* 333-338 (2000).
8. Caceres, D.D., Hancke, J.L. and Burgos, R.A." Use of visual analogue scale measureme*nts (VAS) to asses the effectiveness of standardized* Andrographis paniculata SHA-10 in reducing the symptoms of common cold: A randomized double blind-placebo study". *Phytomedicine***. 6**:217-223 (1999).
9. Anon. Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) 2003, UKMAEC Secretariat, Kuala Lumpur, 1-40 (2003).
10. Abdulelah, H.A.A. and and Zainal-Abidin." *In vivo* anti-malarial tests of *Nigella sativa* (Black Seed) different extracts". *Amerrican Journal of Pharmacology and Toxicology,* **2**(2): 46-50 (2007).
11. World Health Organization (WHO). World Malaria Report 2008, WHO Geneva vii (2008).
12. Nurhidanatasha, A.B. and. Zainal - Abidin B.A.H. "Toxological and antimalarial activities of andrographolide and *Andrographis paniculata* extracts in mice." *Proceedings of Herbal Symposium, Spice and Herbs Paradise Johor Malaysia* 235-239 (2003).
13. Panossian, A., Hovhannisyan, A., Mamikonyan, G., Abrahamian, H., Hambardzumyan, E., Gabrielian, E., Goukasova, G., Wikman, G. and Wagner H. " Pharmacokinetic and oral bioavailability of andrographolide from *Andrographis paniculata* fixed combination Kan Jang in rats and human". *Phytomedicine*", **7**(5): 351-364 (2000)
14. Puri, A., Saxena, R.P. and Saxena, K.C. "Immunostimulat agents from Andrographis paniculata". *Journal of Natural Products,* **56**(7): 995-999 (1993).
15. Nurhidanatasha, A.B. Nurul Izza, N.,Nik Idris, N.Y., Bohari, M.Y. and Zainal-Abidin, B.A.H. "Antimalarial activity of *Andrographis paniculata* and an organometallic ferrocenium tetrachloroantimonate against rodent malaria in mice". *Proceedings of the 7th Symposium of Applied Biology, Kuala Lumpur Malaysia* 78-81 (2003).
16. Misra, P., Pal, N.L., Guru, P.Y., Katiyar, J.C., Srivastava, V. and Tandon, J.S."Antimalarial activity of *Andrographis paniculata* (Kalmegh) against *Plasmodium berghei* NK65 in *Mastomys natalensis*". *Int. J. Pharmacog,* **30**(4): 263-274 (1992).